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ESTROGENIC ACTIVITY OF ISOFLAVONOID OBTAINED FROM LAWSONIA INERMIS

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ABSTRACT

An isoflavonoid has been isolated from the stem extract of Lawsonia inermis Linn. (Lythraceae). The isoflavonoid tested on female albino rats, showed estrogenic activity by increasing uteri weight from 16.5 ± 0.28 mg in control to 34.4 ± 0.69 mg in orally treated rats. Air-dried and powdered stem of Lawsonia inermis was extracted with hot rectified spirit. The extract obtained was evaporated to dryness. The residue obtained was subjected to successive extraction with petrolium ether, benzene, chloroform, ethyl acetate, and methanol. The residue of the methanol extract was subjected to column chromatography over silica gel column with chloroformmethanol mixtures. The 7:3 chloroform-methanol elute was distilled in order to remove the solvent affording pale yellow crystals of compound. The compound has been identified as the isoflavonoid genistein by the joint application of chemical methods and spectroscopic methods (UV, IR, 1HNMR, and 13CNMR). The compound was obtained as a pale yellow mass, which was re-crystallised by methanol, analysed for C15H10O5 m.p, 285-287.The crude extract and methanol solution of the compound on screening by "four day uterine weight assay" showed positive estrogenic activity. Result showed the dose dependent estrogenic activity of compound by increasing uterine weight from 15.6 ± 0.26 mg in orally treated female albino rats (1.25 mg/kg/day for four days).

KEYWORDS: 13CNMR, four days uteri weight assay, EIMS, estrogenic activity, extraction, genistein. 1HNMR, isolation, isoflavone, Lawsonia inermis Linn.

Introduction

Disease and death still continue to be the biggest threat to the human being. No country in this world, however advanced, can claim to conquer all diseases. Although several synthetic drugs are being added to the world of modern pharmacopoeia, still there exists a great concern for the survival of humanity from so many unconquered diseases, which naturally compels the scientists working in the area to face the problem seriously and explore the natural wealth in the form of medicinal plants. The role of bioactive constituents of plants in drug discovery and development is well known. Annual reports of medicinal chemistry show that new approved anticancer and anti-infective drugs include major contributions of natural products (Cheng et al, 1993). Keeping this fact in mind, a phytochemical investigation has been performed of *Lawsonia inermis* Linn (Lythraceae), a plant cultivated all over India that has significant medicinal properties (Chopara et. al, 2002). An isoflavonoid genistein was isolated from the stem of *Lawsonia inermis* and found to possess estrogenic activity. The level of active compound genistein in the leaves of *Psoralea corylifolia* was found 2 g Kg-1 dry weight. In other legumes, Lupinus spp., Vicia faba and Pueraria lobata, the level of genistein was found <400 mg Kg-1 dry weight (Kaufman et. al, 1997).

Material and Methods

Plant material (stem) was collected in October 2006 from University campus, Allahabad, India and specimen deposited in the Natural Product Laboratory, Department of Chemistry, Allahabad (voucher specimen number A-3). The plant material is identified by Professor A. Shrotriya, Department of Chemistry, Government Post Graduate College, Guna, M.P., India. Shade-driedand powdered stem were extracted with aqueous methanol to make a liquid slurry and the mixture is left for seven days. Extract was separated from plant material by filteration, using a buckner funnel by vaccum pump. The methanol extract was concentrated under reduced pressure. The resultant syrupy mass was evaporated so methanol has been removed and crude compound was obtained (Markham 1982). Remaining methanol extract was subjected to successive extraction with petroleum ether (Rankem, HPLC grade, 99.8%), benzene (Rankem HPLC grade, 99.8%), chloroform(Merck HPLC grade 99.0-99.4%), ethyl acetate (Rankem HPLC grade 99.8%) and methanol (Qualigens HPLC grade 99.8%). The methanol extract obtained was fractionated and fraction was tested for positive estrogenic activity. The crude compound obtained by the removal of methanol was subjected to column chromatography in silica gel column and eluted with a mixture of chloroform and methanol in varying proportions. Fractions with adequate solid precipitates were subjected to preparative thin layer chromatography (TLC), fractions with same Rf values were mixed and evaporated to remove solvents. After concentration yellow coloured compound was isolated, which was recrystallised with methanol into needle like yellow crystal, compound. The methanol extract of compound on screening on female albino rats by "four day uterine weight assay" showed positive estrogenic activity (Turner & Hebborn 1971; Thompson 1990). Female albino rats of 21-30 days age and 40 g average weight were selected for the experiments. Five groups of rats were taken for the experiment. One group was kept as control (Q). 10 µg/rat dose of drug (concentrated methanol solution of compound) was given orally with diet to one group (A) 20 µg/rat was given to second group (B) and 50 µg/rat was given to third group (C). After 4 days the rats were sacrificed and their uteri were taken out. Uteri were dehydrated in oven at 100°C for 24 h and then reweighed. Similarly the time of complete vaginal opening was observed and compared with control.

Result and Discussion

Compound analysed for C15H10O5, mp 285-2870C. M+ 270, UVmax (MeOH) 264, 328 nm, IR bands (nujal) 1680, 3340 cm-1. 1 H NMR: _ 7.82 (1H, S, H-2), 7.51 (2H, d *J* = 9 Hz, H-2_and H- 6_), 7.12 (2H, d, *J* = 9 Hz, H-3_and H-5_), 6.55 (1H, d, *J* = 2.5 Hz, H-8), 6.45 (1H, d, *J* = 2.5 Hz, H-6), [M+] 270, m/z 269, 153, 152, 135, 124.

By the joint application of chemical methods (Saxena & Singhal, 1998) and spectroscopic data the compound has been identified as 5,7,4_-trihydroxy-isoflavone genistein. The methanol solution of compound was tested on female albino rats. It showed dose dependent estrogenic activity by increasing uteri weight from 16.5 ± 0.25 mg to 35.2 ± 0.68 mg in control and orally treated rats. Mean uteri weight and number of vaginal openings were recorded in the *Table 1*, showed the dose dependent estrogenic activity of compound in methanol by increasing uteri weight from 16.5 ± 0.28 mg in control to 34.4 ± 0.69 mg in orally treated rats.

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Table 1

Group	Dose in µg/rat	No. of rats	Mean uteri wt in mg	No. of vaginal opening
Q	0.0	5	16.5 ± 0.28 mg	0
A	10	5	18.6 ± 0.56 mg	0
B	20	5	28.0 ± 0.52 mg	3
C	30	5	34.4 ± 0.69 mg	5

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